# Triglycerides



CE

## Reagent for quantitative In-vitro-determination of triglycerides in blood and serum / plasma

**TRI 142**

**Order No.** **TRI 142**
Content: 40 tests

### Method
Enzymatic colorimetric test, GPO-PAP method[1] without considering free glycerol.

The determination may be carried out with blood as well as serum/plasma. Blood is immediately and completely haemolysed by the reagent. The result obtained from blood also refers to plasma.

### Sample material
Capillary or EDTA venous blood.
Serum, haeparinized or EDTA plasma.
Pipette capillary blood immediately into single test cuvette.
Stability of triglycerides in buffer solution:
at + 2 to + 8°C: 8 hours
at + 15 to + 30°C: 4 hours

### Reagents
Contents / concentrations of the ready-to-use solution:
1. Starter reagent (screw caps)
   L-glycerol-3-phosphate-oxidase (GPO) from microorganisms > 3.5 kU/L, Glycerokinase (GK) from bacillus stearothermophilus > 0.9 kU/L, Peroxidase (POD) > 3.5 kU/L, ATP 2.4 mmol/L, 4-Aminophenazone 0.15 mmol/L
2. Buffer solution (pre-portioned in round cuvettes)
   Lipoprotein lipase from microorg. > 7.5 kU/L, 2.4-Dichlorophenol 4 mmol/L, Sodium azide < 0.1 %; Triton X-100 < 1%, PIPES-buffer 50 mmol/L, pH 7.5

### Safety information
The buffer solution (round cuvette) contains sodium azide (<0.1 %) and Triton X-100. Do not swallow and avoid contact with skin and mucous membranes. If desired a safety data sheet will be provided.[3]

### Storage and shelf life
The test reagents can be kept at a temperature between +2°C and +8°C until the expiry date indicated on the packaging. Please take the screw caps out of the container just before the analysis.

### Measurement conditions

| | |
|---|---|
| Measurement device: | Diaglobal Photometer |
| Meas. wavelength: | 520nm |
| Temperature: | Roomtemperature |

The algorithm to compute the analysis result is coded in the above-named photometers.

### Measurement range
20 - 2000 mg/dL (0.2 - 22.8 mmol/L)

### Working instructions
The measurement can be performed as a single or serial measurement (with a balancing of the A(0) counts = blank values).

A. Single measurement

| Pipette into round cuvette: | |
|---|---|
| | Analysis |
| Sample | 10 µL |
| Mix thoroughly. | |

- Select the <**TRI**> test
- Insert analysis cuvette (blank value)
- Screw the cap from PE-bottle onto the cuvette, dissolve the starter reagent by inverting several times
- Press [ON/ENTER]
- Insert analysis cuvette again
- Wait for result

B. Measurement of series (up to 20 samples)

| Pipette in round cuvettes: | |
|---|---|
| | Analysis |
| Sample | 10 µL |
| Mix thoroughly. | |

- Select the <**TRI**> test
- Insert the analysis cuvettes one after another (blank values)
- Screw the caps from PE-bottle onto the cuvettes, dissolve the starter reagent by inverting several times
- Press [ON/ENTER]
- Insert the first analysis cuvette **immediately** again
- Wait for result
- Insert the other analysis cuvettes one after another in the same order as of the blank value measurement
- Results of the respective analysis cuvette can be read immediately

### Quality assurance
For quality assurance we recommend universal control sera from company Roche, www.roche.de:
PreciControl ClinChem Multi 1 / Multi 2 (4 x 5 mL)
Order-No.: 05 947 626 190 / 05 947 774 190
Ref: Roche / Hitachi analyzers, Method: GPO – PAP

### Reference values
For recognition of hypertriglyceridemia the following upper marginal values are recommended.[1]

| | mg/dL | mmol/L |
|---|---|---|
| Suspicious from | 150 | 1.71 |
| Elevated from | 200 | 2.29 |

### Summary
After meals the triglyceride concentration in blood increases heavily since triglycerides, which are absorbed through nourishment, reach relatively fast the blood as chylomicrons. Diagnostic conclusions may only be drawn with values from fasting blood.

Indications / diagnostic significance:[1]
- Early recognition of arteriosclerotic risk
- Classification of hyperlipoproteinemia
- Control of lipid-lowering therapies

Elevated triglyceride counts are also often found with diabetes mellitus, chronic renal insufficiency, liver damage, pancreatitis, and alcoholic excess. They can also be a signal for a disease that has been unrecognised so far.

Today the determination of triglycerides is carried out without exception enzymatically over glycerol that has been released by hydrolytic cleavage.[2] The former widespread UV method, where the NADH decrease used to be measured after a multilevel enzymatic reaction, has been displaced by the GPO-PAP method[4], which the Diaglobal test is also based on.

### Measurement principle

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Free fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + ADP$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone Phosphate} + H_2O_2$$

$$H_2O_2 + \text{2,4-Dichlorophenol} + \text{4-Aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Quinonimine dye}$$

GK = Glycerokinase, GPO = Glycerol-3-phosphate-oxidase

The concentration of the quinonimine dye is a measure for the triglyceride concentration in blood and serum/ plasma respectively and is measured photometrically at 520 nm.
Reaching the end point of the reaction is identified automatically by the device itself.
Free glycerol in serum, whose concentration corresponds to a triglyceride value of 10 mg/dL if the person is healthy, is measured, too.

### Performance parameters
**Specificity / interferences[1,5]**
Elevated values because of free glycerol, no interferences due to ascorbic acid in physiological concentrations (< 30 mg/dL) and bilirubin (< 10 mg/dl) as well as due to a low and high haemoglobin level. Interferences due to pharmaceuticals[5]: lowered values due to acetylsalicylic acid (> 75 mg/L), no interferences due to methyldopa and novaminsulfon in therapeutic concentrations.

**Inaccuracy**
The reproducibility was checked using human and control samples.

| In series [n = 20] | Average [mg/dL] | Standard deviation [mg/dL] | VK [%] |
|---|---|---|---|
| EDTA blood | 102 | 4.0 | 3.9 |
| Serum 1 | 125 | 3.1 | 2.5 |
| Serum 2 | 197 | 3.5 | 18 |
| From day to day [n = 20] | Average [mg/dL] | Standard deviation [mg/dL] | VK [%] |
| Serum 1 | 120 | 4.0 | 3.2 |
| Serum 2 | 199 | 4.1 | 2.1 |

### Analytic sensitiveness
Lower detection limit: 20 mg/dL (0.2 mmol/L)

### Comparison of methods
A comparison of the Diaglobal test TRI 142 (y) and a commercially available test (x) based on the GPO-PAP method as well as a comparison of values that have been determined with blood (y) and plasma (x) of the same proband resulted in the following correlation data according to the Passing/Bablok[6] process:

a) Serum
y = 1,019x - 5,27
r = 0,999
n = 44

b) EDTA blood / plasma
y = 1,002 + 0,82
r = 0,995
n = 73

Concentration range: 45 - 1500 mg/dL

### Information on disposal
Waste code number 180106:
Vials with reagent are considered hazardous waste. Do not allow reagent to reach surface water or sewage system.
Dispose of in accordance with official regulations.
Non-contaminated and completely empty packaging can be recycled.
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### Bibliography
1. Thomas L. Labor und Diagnose. 4th edition. Marburg: Die Medizinische Verlagsgesellschaft 1995: 203
2. Klotsch SE, McNamara JR. Triglyceride measurements: a review of methods and interferences. Clin Chem 1990; 36:1605
3. http://www.diaglobal.de/de/service/downloads/index.html
4. Fossati P, Principe L. Clin Chem 1982; 28:2077
5. Sonntag O. Arzneimittelinterferenzen. Stuttgart, New Yorck: Thieme Verlag, 1985:37
6. Passing H, Bablok W. A new biometric procedure for testing the equality of measurements from two different analytical methods. J Clin Chem Clin Biochem. 1983; 21:709